Laboratory of Environmental Mutagenesis:
Summary Statement

by H. V. Malling

The primary objective of the Institute's program in mutagenesis is focused on evaluation and definition of risks to both human somatic and germinal tissue resulting from exposure to genetically active environmental chemicals. The Laboratory of Environmental Mutagenesis was organized late in FY 1972. Because of the worldwide shortage of resources for the rapid development of research in various problem areas associated with environmental mutagenesis, staff members have used a variety of approaches to accomplish its objective. These include:

(1) Development of an intramural research program divided into eight main areas. Work on particular projects has been carried out by different mechanisms: in-house research by staff scientists, support of work at other institutions in the collaborative research program with staff scientists as project officers, and developing an announcement in collaboration with the extramural program office to solicit grant proposals in specific emphasis areas of environmental mutagenesis.

(2) Development of a coordinated national program in environmental mutagenesis. A variety of mechanisms have been initiated, including: participation in the DHEW Subcommittee on Environmental Mutagenesis (SEM), developing workshops and conferences in special emphasis areas, developing collaborative programs with other institutes within NIH and with other agencies, developing programs within the Environmental Mutagen Society in which staff members serve as officers, councillors, or members, and developing collaborative studies on genetically active environmental chemicals to rapidly develop a data base to assist in evaluating the risk of exposure to man.

(3) Development of better communication and coordination at the international level. Staff members are associated with several international programs, including a U.S.-U.S.S.R. Environmental Protection Agreement, participation in international workshops and conferences, collaboration with IARC (Lyon) on the mutagenicity of chemical carcinogens.

(4) Development of better mechanisms for information exchange, including support of the Environmental Mutagen Information Center (EMIC) and serving as associate editor or members of the editorial board on journals which publish research in the area of environmental mutagenesis

(5) Development of better mechanisms for training young scientists for research careers in environmental mutagenesis at the predoctoral level and the postdoctoral level.

Intramural Research Program

The intramural staff has been organized into seven programs. The major research accomplishments in each program are as follows:

The Microbial Genetics Section has made considerable progress in the development and improvement of simple and comprehensive tests for gene mutations and other types of genetic damage in microorganisms. Dr. Zeiger has attempted to correlate the mutagenicity of various procarcinogens using rat liver homogenate as activation system with measurement of the enzyme activity in the same aliquots. Together with Dr. Chhabra, he also is trying to study the relationship between the mutagenic activation in the intrasanguineous host-mediated assay and activation *in vitro* using bacteria as indicator organisms.

Drs. Frezza and Zeiger are using two different yeasts in the intrasanguineous host-mediated assay. Dr. Malling and Mr. Claxton have developed an instant computerized data acquisition system for the short-term testing of mutagenic activity.

Yeast is the only system for studying mitotic recombination. Unfortunately, most yeast strains are rather impermeable. Dr. Callen is using crystal violet to isolate more permeable strains of yeast. He is

also studying the formation of toxic and mutagenic compounds by ultraviolet irradiation of fuel oil on top of water.

Dr. Scott has been working especially on developing a system in *Aspergillus nidulans* for detection of 50 plus genetic events. These events range from point mutations, chromosome aberrations, and mitotic recombination. Dr. Scott has tried also to develop *Salmonella typhimurium, E. coli, Neurospora crassa,* and *Aspergillus nidulans.* None of these systems are as sensitive as Tradescantia.

Mr. Harvey has studied the tritiated water as a mutagen in the *ad-3* mutation system.

Dr. Ong has developed a spot test system for detecting reverse mutations at the *ad-3* locus of *Neurospora crassa*. Various tester strains have been selected that exhibit sensitivity to various types of chemical mutagens. This rapid assay will complement the *ad-3* forward mutation system earlier developed by Dr. de Serres which has been used by Drs. Ong and de Serres to test the mutagenicity of several chemical carcinogens and a wide variety of environmental chemicals. These studies are designed primarily as structure-function analyses to determine the correlation between types of genetic damage induced by compounds of similar structure. Many chemicals do not exert mutagenesis by themselves but can be converted to mutagenic metabolites. Drs. Whong and Ong are using the *ad-3* system to study the activation of dimethylnitrosamine to mutagens in the host-mediated assay and are comparing the results from different tissues and species.

Drs. de Serres, Ong, and Inoue also are continuing studies on repair-deficient strains of Neurospora. In a comparison between the normal heterokaryon and one homozygotic for repair-deficient mutants for five mutagens, it was found that they all caused more killing in the repairless strains than in the normal strain, but only those chemicals which react covalently with the DNA. Mr. Harvey also has extended the study on the repairless mutants in Neurospora and has shown that *upr-1* and *uvs-2* are not in the same pathway of repair.

Drs. Maier, Chhabra, and Malling have focused their attention on the activation systems which occur in the testicular cells and found that nitrofurazones are activated to mutagenic metabolites.

Great progress has been made within the Mammalian Biochemical Mutagenesis Program, where Dr. Soares was able to isolate biochemical mutations in mice using the biochemical specific locus mutation system. Dr. Soares used TEM as a model mutagen and has isolated more than 10 mutants. Dr. Valcovic has continued the study of the ^{60}Co-induced biochemical mutants. This mutation system will be expanded in the future to cover many more parameters. Dr. Lee is working on a system for detection of heat-instability mutations and mutations which affect specific activity of enzymes. Mr. Burkhart is developing the techniques and computer programs for the high speed centrifugal enzyme kinetic analyzer (MCFA). In order to understand the limitations of these techniques, Dr. Pegoraro is studying the naturally occurring polymorphic enzymes in mice.

The Biochemical Genetics Group is new and is a very important addition to the Laboratory of Environmental Mutagenesis. The group will study the enzymes and mutant enzymes in detail in order to elucidate the molecular mechanism of mutagenesis in mammals. Dr. Li will characterize the various isozymes and mutant proteins and correlate the amino acid differences and antigenic determinants of lactic dehydrogenase in mammals. Dr. Marciniszyn will concentrate on the sperm-specific lactate dehydrogenase in mammals, and Dr. Chen will characterize the mutant proteins from mammalian somatic cells.

The studies within the mammalian genetics program are concerned primarily with the detection of induced genetic damage in mice, investigations of the transmissibility of the damage to the progeny, and the long-term effect of mutations and chromosomal aberrations on the development of the embryo.

Drs. Sheridan and Soares are comparing the induction of dominant lethal mutations and translocations induced in a wide variety of inbred strains of mice after treatment with a given dose of triethylenemelamine. The objective of this work is to develop a battery of tester strains ranging from very sensitive to very resistant which will better mimic the range of variation that we can expect in the heterogeneous human population. They also are studying the induction of recessive lethal by chemical mutagens. This is an especially important study, since it can be assumed that each human carries five or six recessive lethal mutations, and not much is known about their effect on fitness of the individual. In order to study the apparent lack of response of mouse spermatogonial stem cells to mutagenic treatment, Drs. Sheridan and Soares have used combined treatments of radiation and chemical mutagens and are investigating whether or not pretreatment with irradiation will sensitize spermatogonia to induction of mutations by chemicals.

A study to detect induced genetic changes in both somatic and germ cells is being carried on by Drs.

Soares and Sheridan. Both lymphocytes and sperm from chemically treated males are being examined for chromosomal aberrations. The aim of this approach is to use somatic cell mutagenesis as a predictor for germ cell effects. The success of this program could greatly reduce the cost and time of mammalian mutagenicity testing.

Drs. Burki and Sheridan have studied chromosome aberrations in early mouse embryos after mutagenic treatment of parents. While dominant lethal effects have been induced by several chemicals, the genetic causes have not been elucidated. This research seeks to determine the relative contribution of chromosome aberrations to this class of dominant lethal events.

Drs. Michelmann and Sheridan have studied the effect and unbalanced genomes on the development of the early embryos. These studies are especially important for understanding Down's Syndrome in humans.

In the Somatic Cell Genetics Group, the mechanism for induction of mutations in mammalian cells *in vivo* and *in vitro* are being sought. Drs. Malling and Ansari are utilizing the various hemoglobins of the inbred strains of mice which differ in as many as nine amino acids. Attempts to produce specific antibodies to these variant hemoglobins are carried out. The antibodies will be coupled with fluorescent molecules. Using these labeled antibodies, mutations can be detected in red blood cells. Other systems are being developed by Dr. Malling which are based on detection of mutations in sperm specific enzymes using histochemical techniques. At the present time we have developed histochemical stains for succinyl dehydrogenase, α-glycerolphosphate dehydrogenase, and lactic acid dehydrogenase and have found two inhibitors for the first and last enzyme. Mr. Dix is attempting to develop an automatic screening system for detection of these variant cells down to the level of 10^{-6}.

Dr. Huang is developing a gene mutation detection system in human diploid fibroblasts. Forward mutations will be detected at a sex-linked locus (HGPRT) and an autosomal locus (AGPRT). At the same time he is measuring the amount of adduct formed after reaction of the mutagen with the DNA. This will quantitatively give the efficiency of the chemical as a mutagen. Dr. Huang also is studying the effect of particular matter such as asbestos on cell death and mutation induction and has found that the toxicity is directly related to the amount of phagocytosis. In order to better understand the mutation expression, he is correlating the thioguanine resistant mutation frequency with the half-life of HGPRT in the cells.

A predominant part of the effort within the Population Genetics Program concerns itself with determination of the frequency of newly arisen genetic variation and its effect on the fitness of natural populations. In order to provide the background, Dr. Voelker is studying naturally occurring alleles of enzyme loci which result in no enzyme activity at all. To do this he has constructed a strain which is heterozygotic at 22 loci.

Drs. Langley and Smith have extended this study to see if there is any association among the various biochemical polymorphism and the physiological or developmental effect of these genes. They found very little nonrandom association.

Drs. Langley and Shah have studied the variation of the mitochondrial DNA within the species of Drosophila; this will lead to a better understanding of the role of the mitochondria DNA in the cellular development.

The Mutagenesis Testing Program is a new function for LEM established by this year's budget, which will be carried out under the collaborative program and coordinated by Dr. Valcovic. While it is not part of the intramural research program, it can be considered to be an extension of the in-house effort.

Until recently, mutagenicity testing was done on preselected compounds in a manner in which the testing laboratories knew the identity of the substances under test and the "expected" results, i.e., positive for compounds selected because of their carcinogenicity and negative for food additives. There is no completed study in which substances were tested blind using a standardized protocol. Also, little attention has been placed on reproducibility and variability within and between laboratories. These aspects are currently under investigation in microbial systems by NCI but the results will not be available for 1–2 years.

In the NIEHS testing program a large number of substances will be tested in a blind study. This will involve the use of a hierarchy of tests proceeding from rapid screening in simple, highly sensitive microbial systems, through *in vitro* mammalian systems, up to whole mammal tests.

Collaborative Research Program

In the Collaborative Research Program, most of the contracts fall naturally within five areas: (1) development of systems for monitoring of the human population for mutations, (2) development of risk extrapolation systems to predict induced mutation rate in the human population, (3) development of short-term test systems for mutagenicity, (4) monitoring of the human environment for mutagenic compounds, and (5) dissemination of

mutagenesis information.

Mutation Monitoring Systems for the Human Population

In a contract at the University of Washington, an attempt is being made to develop a simple system to measure point mutations in readily accessible human somatic cells. This assay is based on changes in the expected frequency of human red blood cells containing hemoglobin S rather than normal hemoglobin. Specific antiserum is being developed to detect mutations which produce hemoglobin S. By conjugating the antiserum with different colored fluorescent dyes, mutant RBC's will fluoresce.

Risk Extrapolation Systems

Recessive Lethal Mutations. In a contract at The Jackson Laboratory, progress has been made toward the development of a new mouse strain carrying multiple chromosome inversions to make it possible to detect gene mutations (recessive lethal mutations) in the chromosome regions covered by the inversions. One of the 39 inversions thus far recovered has been used in tests to detect x-ray induced recessive lethal mutations to demonstrate the general utility of this approach. Fourteen inversions have been mapped and are now being combined into a single mouse strain. Some of the combinations have lead to sterility.

Biochemical Mutations. A contract with The Jackson Laboratory involves the incorporation of 13 additional mouse enzyme alleles into the C57BL/6J strain. This will directly complement the electrophoretic assay system developed at our Institute and will significantly enhance the sensitivity of the system to detect biochemical specific locus mutations.

In a contract with Research Triangle Institute, the progeny from treatment of the male parent with TEM is presently being screened with a role of 800 mice per month. Many different types of mutations, such as esterase mutations, have been found.

Polygenic Mutations. A contract with the Georgia Institute of Technology attempts to develop a polygenic assay for the detection of point mutations in mice. By studying changes in the means and variances of characters determined by many genes, it should be possible to detect genetic changes which do not express major phenotypic effects.

Dietary Deficiency Effect on Mutation Rate. In a contract with Knox College, studies are being conducted in *Drosophila melanogaster* to determine the effects of dietary deficiency on the sensitivity to mutagenic treatment. Preliminary results have indicated that deficiencies in choline and cholesterol increase the amount of genetic damage observed following treatment.

Short-Term Tests for Mutagenicity

Computer System for Short-Term Tests. A contract at New York Medical School attempts to develop, in collaboration with DCRT in Washington, a computer system for assisting, advising, and collecting the data from short-term tests. The system is now working very well.

Testing of Neurospora Mutants. We have continued to support a contract at Miles Laboratory to perform the genetic analysis of *ad-3* mutants recovered from experiments on haploid strains of two-component heterokaryons. During the past two years we have also been using repair-deficient strains of Neurospora, as the genetic characterization performed in this manner has shown striking qualitative differences between wild-type and excision repair-deficient strains.

Monitoring of the Human Environment for Mutagenic Compounds

By interagency agreement, we are supporting baseline studies to determine the mutagenicity of airborne chemical pollutants and other gases used by industry. Various clones of the plant *Tradescantia paludosa* are being developed to select one particularly sensitive clone for assays of the genetic activity of weak mutagens. Field studies have been done in collaboration with NERC-EPA to compare the effects of exposure to the same airborne pollutants under laboratory and field conditions, and positive results have been obtained from some heavily polluted areas.

Collection and Dissemination of Mutagenesis Literature

During the past year we have continued to support EMIC (Environmental Mutagen Information Center) by interagency agreement at the Oak Ridge National Laboratory. This center has over 19,000 bibliographic entries in its data banks and contains as a unique world-wide resource for information in the area of environmental chemical mutagenesis.

National Programs

To develop better coordination in environmental

mutagenesis and to provide perspective for the intramural scientific staff in problem definition and resolution in the rapidly developing field, a variety of mechanisms have been developed.

SEM (DHEW Subcommittee on Environmental Mutagenesis)

Intramural staff members have continued participation with at least 20-30 representatives from DHEW and other government agencies to develop better communication within DHEW as well as between other agencies with programs in environmental mutagenesis. The Panel meets monthly for discussion, and minutes are recorded to provide the basis for furthering communication within the various member institutes and agencies. A subcommittee was formed of SEM to develop the government's general position on mutagenesis testing. Dr. Valcovic served as Executive Secretary of this group and Drs. Malling and Zeiger were working members. During the development of the document, several open meetings were held at NIH. The document was finally turned over to Dr. Rall in April 1977. This is probably the most important part of the SEM work in FY 1977.

Workshops and Conferences

In collaboration with The Jackson Laboratory in Bar Harbor, Maine, Dr. Sheridan organized a workshop to "Design New Methods for Assessing Mutations in Mammalian Systems," which was held on October 6-8, 1976. The proceedings will be published in GENETICS.

Collaborative Programs with Other Institutes within DHEW and with Other Government Agencies

NCI. In collaboration with staff scientists at NCI, a collaborative research program has been persued to determine the correlation between carcinogenic and mutagenic activity of chemical carcinogens and non-carcinogenic structural analogs. This has also involved development of mechanisms to review and evaluate performance of the contractors as well as the planning of workshops to evaluate the accomplishments of the overall program. In collaboration with staff members of the In Vitro Carcinogenesis Program, new plans have been made to validate microbial and mammalian tests for mutagenicity for use as prescreens in the carcinogenicity test program.

FDA. In collaboration with staff scientists in the Bureau of Foods, NIEHS staff scientists have participated in the successful development and review of collaborative research programs to develop common methods for screening and computerizing data handling.

EPA. In collaboration with staff scientists in the Office of Pesticide Program, NIEHS staff scientists have participated in the development of collaborative research programs to perform mutagenicity tests on pesticides.

NSF and Other Agencies. Staff scientists have participated in the review of proposals for contract work and in site visits to select suitable contractors for research in the area of mutagenicity testing.

International Program

Staff members are associated with a wide variety of international programs with individual countries as well as with international agencies and programs.

U.S.-U.S.S.R. Environmental Protection Agreement

Drs. Langley and Valcovic visited several genetic laboratories in the Soviet Union in September 1976 to discuss future collaboration under this agreement. The fourth workshop on Basic and Practical Approaches to Environmental Mutagenesis and Carcinogenesis was held in San Francisco, California, in April 77. The program was focused on research directed towards developing better screening systems to detect mutagenic and carcinogenic activity as well as methods suitable for genetic monitoring of the human population.

Collaboration with IARC (Lyon)

During FY 1977 a new program was continued which involves participation between the National Institute of Environmental Health Sciences staff scientists, EMIC personnel and IARC scientists to translate mutagenicity data of known chemical carcinogens. Staff members also are participating in a series of workshops organized by the IARC staff to evaluate the correlation between carcinogenic and mutagenic activity and the possibility of using short-term tests for mutagenic activity to predict potential mutagenic and carcinogenic activity in man.

U.S.-People's Republic of China

In connection with the expanded program of scientific exchange with the People's Republic of China, a Chinese delegation visited the Laboratory of Environmental Mutagenesis in September 1976.

International Symposia, Workshops, Meetings

Dr. Malling participated in the meeting of the German Environmental Mutagen Society, held July 1-2, 1976. The meeting of the International Environmental Mutagen Society will be held in Edinburgh, Scotland, in July 1977; and Drs. Malling, Valcovic, and Zeiger will participate. Dr. Malling will be a lecturer at the EMBO Course in Mammalian Genetics in Harwell, England, in September 1977.

Information Exchange

EMIC (Environmental Mutagen Information Center)

The Institute has continued to support the work of EMIC to collect, organize, and disseminate information on environmental chemical mutagens. The Center not only processes requests for information from various government agencies, but is now becoming well known abroad. During the past year, EMIC's data file reached 19,000 bibliographic entries.

Journals

Staff members serve as associate editors, assistant editors or members of editorial boards of MUTATION RESEARCH, CANCER RESEARCH, ENVIRONMENTAL HEALTH PERSPECTIVES. JOURNAL OF TOXICOLOGY, and, REVIEWS IN GENETIC TOXICOLOGY. This makes it possible not only to promote publication of papers dealing with various research areas of environmental mutagenesis but also to promote development of new methods for publication.

Training Programs

The great shortage of scientists to do research in the area of environmental mutagenesis both in the United States and abroad has provided incentive for staff scientists to develop a training program at both the predoctoral and postdoctoral levels. During the past year, we have provided facilities for research for three predoctoral students from the University of North Carolina at Chapel Hill. At the postdoctoral level, staff scientists include 16 visiting fellows from ten foreign countries.

In conclusion, during the past year we have been successful in recruiting key personnel for our intramural program, have continued cooperative programs with other Laboratories in the Institute, have coordinated program development of the Laboratory with complementary programs in other Federal agencies, and have established lines of communication with newly developing scientific organizations for environmental mutagenesis abroad. Along with the organization of workshops and conferences, these approaches will be used to stimulate research and development in this rapidly developing area of environmental health science.